SUMMARY AND CONCLUSIONS

The method of Rubin, *et al.* (1), using the immature mouse for the bioassay of estrogens has been modified for the assay of a long acting estrogen, estradiol 17-cyclopentylpropionate (ECP). The modifications consist of administering the hormone in a single subcutaneous dose, removing the uteri 72 hours later, and using uterine weights as responses. The results were analyzed according to U.S.P. XVI (3) for a 2×2 balanced assay. When eight mice were used on each dose, the average log confidence interval was 0.2333.

Similar results were obtained by injecting ECP in single or divided doses. This was not the case with estrone or estradiol which were much more effective when multiple injections were given.

It is suggested that the method described for the assay of ECP may be utilized as a rapid bioassay for other long acting estrogens. It may also be used for identification of long acting estrogens and to differentiate between those that are either long or short acting.

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Liquefaction Time of Rectal Suppositories

By IVO SETNIKAR and SERGIO FANTELLI

An apparatus reproducing the conditions of the environment of the rectum is described. With this apparatus, the liquefaction time of rectal suppositories, either with fatty or water-soluble bases, can be measured. The liquefaction times found for a series of suppositories with fatty bases and with so-called water-soluble bases are reported. It is proposed that a maximum limit should be established for the liquefaction time of rectal suppositories carrying drugs with general action.

The United States Pharmacopeia XVI defines suppositories as "solid bodies of various weights and shapes, adapted for introduction into orifices of the human body and usually melting, softening, or dissolving at body temperature."

However, no method is described for testing these characteristics on the whole suppository. The method for the determination of the melting temperature of fatty (class II) substances is useful for suppository bases but not always applicable to the nonhomogeneous drug-base mixtures of which suppositories are often made. Since the drugs present may alter the melting point (m. p.) of the bases and since the m. p. of the drug-base mixtures may change considerably over a period of time (1), the determination of the m. p. of the suppository base alone is clearly not sufficient for the control of the characteristics mentioned in the U.S.P. definition of suppositories.

Several researchers have felt the need and proposed methods by which these characteristics, and particularly the m. p. can be controlled on the whole suppository. These methods can be classified into two groups: (*a*) m.p. determinations in a dry environment (2, 3) and (*b*) m.p. determinations on suppositories in a water bath (4–7).

With both kinds of methods the m.p. is measured in an environment very different from that of the rectum, for the latter is neither anhydrous nor aqueous. Both give useful information on fatty bases only, because almost all of the so-called water-soluble bases melt at a temperature above body temperature with the first technique, while they dissolve independently of the temperature with the second. Further, with neither method is it possible to record a very important datum, i. e., a representative time of melting, softening, or dissolving at body temperature.

The knowledge of this time is essential in suppositories which include a drug for a general action and must, therefore, be absorbed. Indeed, a suppository which takes too long to liquefy may be expelled before liquefaction, together with the drug it includes. Beside this, it may exert a mechanical irritant action on the rectal ampulla even if the base and the drug, *per se*, are not irritant. The U. S. P. should,

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therefore, recommend, for suppositories including a general-acting drug, a maximum liquefaction time, just as it fixes maximum times for the disintegration of tablets. Also, as in the case of the latter (U. S. P. XVI, p. 830), it should emphasize that "sure disintegration (or rather sure liquefaction) is an essential attribute for suppositories including a general acting drug."

Obviously, if an apparatus is to give a reliable indication on the liquefaction of rectal suppositories, it must reproduce the mechanical and physicochemical conditions of the rectum as faithfully as possible.

The human rectum, or distal portion of the intestine, is 12–14 cm. long and has a maximum diameter of 5–6 cm. The following conditions are present: (*a*) In healthy subjects the average temperature (8) is 36.9° (from 36.2 to 37.6°). (*b*) Water is not present in the liquid state; the feces are semisolid and contain 77–82% water (9). (*c*) Water may issue from the blood through a process of osmosis. Although the rectal mucosa tends to absorb water and transfer it to the blood, it may be regarded as a semipermeable membrane since it also permits the passage of water in the opposite direction, provided that there are osmotic forces to attract it. This process is, however, antiphysiological and may be both irritating and painful. (*d*) There are practically no peristaltic movements (10) to churn and mix up the contents of the ampulla of the rectum. (*e*) The pressure on the contents of the rectum is, according to posture, from 0 to 50 cm. water (10, 11). (*f*) Feces may be present.

Nearly all these conditions (temperature, heat transmission, semipermeability of the walls, pressure) may be reproduced in a semipermeable cellophane dialyzer tube immersed in a water bath at 37.0°.

EXPERIMENTAL

Apparatus.—The apparatus (Fig. 1) consists of a glass cylinder with an external diameter of 50 mm., narrowing down to 22 mm. at either end for a length of 30 mm. The cylinder is fitted with two connections through which water circulates from a circulating water pump, thermoregulated to 37.0°.

The apparatus is prepared in the following way: a 34–35-cm. length of cellulose dialyzer tubing, size inflated diameter 1 1/8 in.,[1] is moistened, opened, and placed in the glass cylinder. The tube is drawn out of either end of the cylinder and secured thereto with two elastic bands. Then the rubber tubes of the thermoregulated pump are attached to the connections of the apparatus and water is made to circulate in it. The apparatus is raised or lowered, keeping it vertical, until the level is found at which the lower half of the cellophane tube is collapsed and the upper half gaping. At the level at which the tube starts to close in, the hydrostatic pressure of the water in the apparatus is about zero.

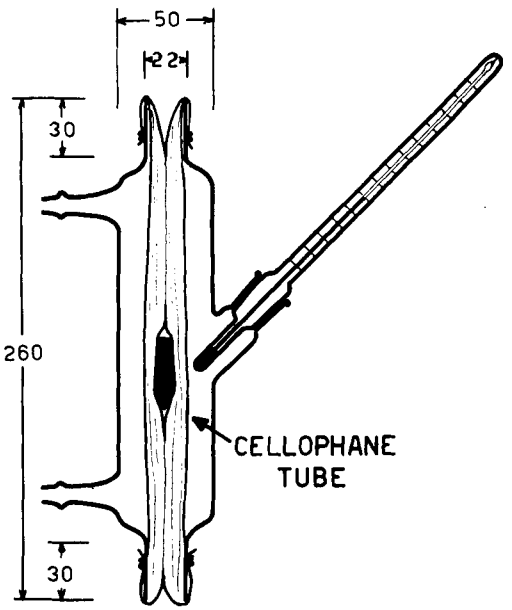

Fig. 1.—Apparatus for measuring the liquefaction time of rectal suppositories; the dimensions are in millimeters. The thermometer scale is divided into tenths of a degree and a scale ranging from 32 to 45° is adequate.

When the thermometer reaches a stable temperature of 37.0°, a suppository is dropped in, the apparatus is lowered about 30 cm., and measurement of the liquefaction time begins.

If the suppository contains a small quantity of drug, or if the drug is dissolved in the base, one can gauge to a high degree of accuracy the moment in which the suppository has entirely liquefied. When the suppository contains large quantities of undissolved drugs, e.g., 0.5 Gm. of aminophylline, it is more difficult to determine the moment of complete liquefaction. In this case it may be helpful to raise the apparatus every 2–3 minutes so as to open up the cellophane tube at suppository level in order to facilitate dispersion of the material already liquefied. Another device is to place a metal spiral, with a diameter of about 5 mm. and a pitch of 3–5 mm., near the suppository in order to promote upward and downward dispersion of the liquefied material. In the most difficult cases, the difference in the assessment of the moment of complete liquefaction by independent observers does not exceed 10–30 seconds.

The apparatus can also be used for the determination of the m.p. of suppositories made by water-insoluble bases. For this purpose the temperature is raised at a speed of 1° every 10 minutes until the suppository melts.

When the experiment is over, the apparatus is raised until the cellophane tube gapes, the inner walls of the tube are washed with water containing a detergent, then with distilled water, and allowed to drip for a few minutes. The apparatus is then ready for use again.

[1] Fisher Catalog No. 8–667.

Results.—Suppositories consisting of commercially available bases, the compositions of which are reported by Pennati and Steiger-Trippi (12), were prepared and kept at room temperature for 1–6 months. The liquefaction time was then measured and the results are given in Table I.

The error of observation was determined by repeating the measurements of ten identical suppositories. For the theobroma oil base the follow-

TABLE I.—MELTING POINT BY THE METHOD OF BOGS AND LIQUEFACTION TIME AT 37.0°

Base	M. P., °C.	Liquefaction Time, min.	Supplier
		Fatty bases	
Theobroma oil	34	6.09	
Caol TR	39	[a]	Sarn, Novara, Italy
Caol TR 38/40	43	[a]	Sarn, Novara, Italy
C.B.S.A. "N"	37	8	Calvè, Delft, Holland
Dehydag III	39	[a]	Deutsche Hydrier-Werke
Dehydag IV	38	[a]	Deutsche Hydrier-Werke
Estarinum A	37	8.58	Edelfettwerke, Hamburg, Germany
Estarinum B	38	30	Edelfettwerke, Hamburg, Germany
Estarinum B B	36	6	Edelfettwerke, Hamburg, Germany
Estarinum C	40	[a]	Edelfettwerke, Hamburg, Germany
Estarinum D	45	[a]	Edelfettwerke, Hamburg, Germany
Estarinum P1	37	10.66	Edelfettwerke, Hamburg, Germany
Imhausen E	38.5	[a]	Imhausen Co., Witten
Imhausen ET	39	[a]	Imhausen Co., Witten
Imhausen H	36	9	Imhausen Co., Witten
Imhausen OG	36.5	7.42	Imhausen Co., Witten
Imhausen W	36.5	10.25	Imhausen Co., Witten
Imhausen WN	39	[a]	Imhausen Co., Witten
Lipomassa C.F.M.	39	33	C.F.M., Milano, Italy
Neo Suppostal	41	75	Medifarma, Milano, Italy
Suppostal ES	45	[a]	Medifarma, Milano, Italy
Suppocire A	37	10.5	Gattefosse, Lyon
Suppocire B	38	[a]	Gattefosse, Lyon
Suppocire C	39	[a]	Gattefosse, Lyon
Suppolanol	35	5.33	Esperis, Milano, Italy
Vi-Tin 136	37	8	Treves, Torino, Italy
Vi-Tin 138	39	[a]	Treves, Torino, Italy
		Water-soluble bases	
Carbowax 1540	46	18	Union Carbide, New York
Carbowax 4000	59	30	Union Carbide, New York
Carbowax 6000	62	45	Union Carbide, New York
Carbowax 6000 + 20% H_2O	54	27	Union Carbide, New York
Glycerinated gelatin, U.S.P.	50	32	
Idromassa C.F.M.	60	40	C.F.M., Milano, Italy
Idropostal	60	37	Medifarma, Milano, Italy
Idropostal 90% + liquid Idropostal 10%	58	35	Medifarma, Milano, Italy
Idropostal G 60% + Glycerol 40%	52	33	Medifarma, Milano, Italy
Idropostal M 90% + H_2O 10%	60	35	Medifarma, Milano, Italy
Idrorectonal H	54	[a]	Frat. Giacomini, Milano, Italy
Idrorectonal HL	41	[a]	Frat. Giacomini, Milano, Italy
Idrorectonal W	55	[a]	Frat. Giacomini, Milano, Italy
Massa Neutralis C.F.M.	39	40	C.F.M., Milano, Italy
Myrj 52	51	31	Atlas Powder Co.
Neutril	59	39	I.C.V., Como, Italy
Tween 61	39	[a]	Atlas Powder Co.

[a] Softening after 10–20 minutes, no liquefaction during 1 hour.

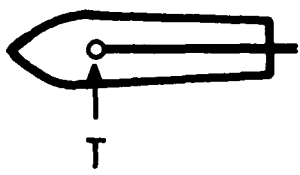

Fig. 2.—Actual shape and size of the suppositories used. *T* indicates the position of the thermocouple inserted in the suppository to measure the speed of heating.

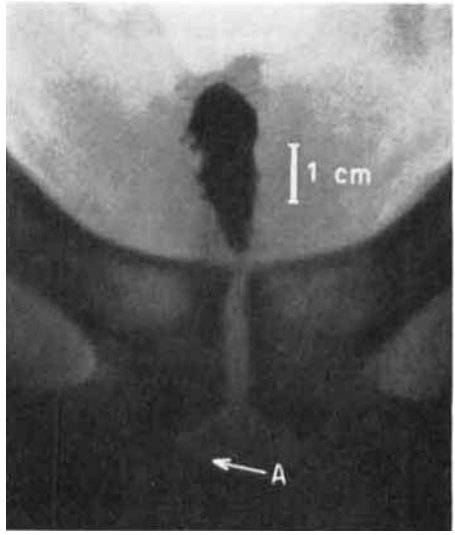

Fig. 3.—Roentgenograph of the pelvic inlet of 34-year-old man, in the supine position, 5 minutes after insertion of a type A suppository. The suppository has completely disintegrated. Arrow *A* marks the position of the anal orifice, to which in this and in the following figures, a lead strip piece was juxtaposed. The dimension of 1 cm. on the plane of the suppository is also marked. The size and shape of the suppository were the same as in Fig. 2.

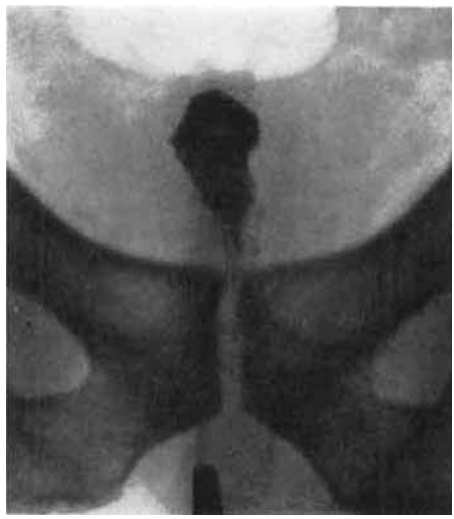

Fig. 4.—Type A suppository, 10 minutes after insertion. The contrast medium tends to spread and rise up the rectum.

ing results were obtained: average liquefaction time 245 seconds, standard deviation ± 10 seconds (4.1% of the average time), standard error 3.2 seconds (1.3% of the average time). Comparable percentage standard deviation and errors were obtained for other bases. The data naturally depend, to some extent, upon the size and shape of the rectal suppositories used. The ones used in these experiments are reproduced in their actual size in Fig. 2.

To check whether the values found by the *in vitro* method were representative of what actually occurs in the ampulla of the human rectum, two types of suppositories were prepared with the following compositions: *Type A Suppositories (Fatty Base).*—theobroma oil, 1.6 Gm.; barium sulfate, 0.5 Gm.

Type B Suppositories (Water-Soluble Base).—Carbowax 1540, 0.8 Gm.; Carbowax 6000, 1.1 Gm.; Polyethylene glycol 400, 0.5 Gm.; barium sulfate, 0.5 Gm.

These suppositories were of the size and shape represented in Fig. 2. The liquefaction time of these suppositories was tested on five healthy male subjects kept throughout the experiment in a supine position. The pelvic inlet was roentgenographed at set intervals. The pictures obtained in one of these experiments are reproduced in Figs. 3–9 and the results summarized in Table II. The agreement between the *in vitro* and *in vivo* results is sufficiently satisfactory to show that the proposed method represents the course of events within the ampulla of the human rectum.

TABLE II.—LIQUEFACTION TIMES *In Vitro* AND *In Vivo*

	Softening Temp.,[a] °C.	M.P.,[a] °C.	Liquefaction Time *in vitro* min.	Liquefaction Time in the Human Rectum, min.
Suppository A	30	34	4.5	between 3 and 7
Suppository B	52	58	40	between 30 and 50

[a] Determined by a modified Bogs method.

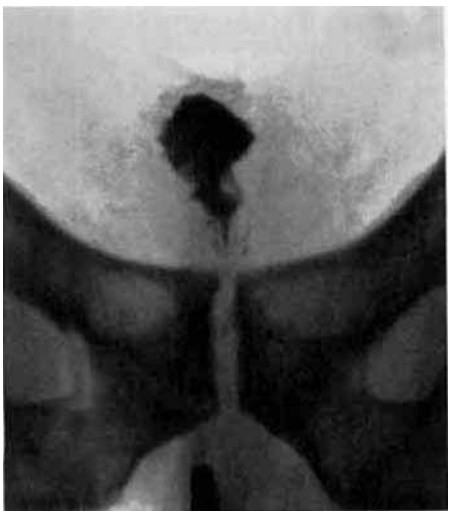

Fig. 5.—Suppository type A, 15 minutes after insertion. The upward movement and spread of the contrast medium are even more marked.

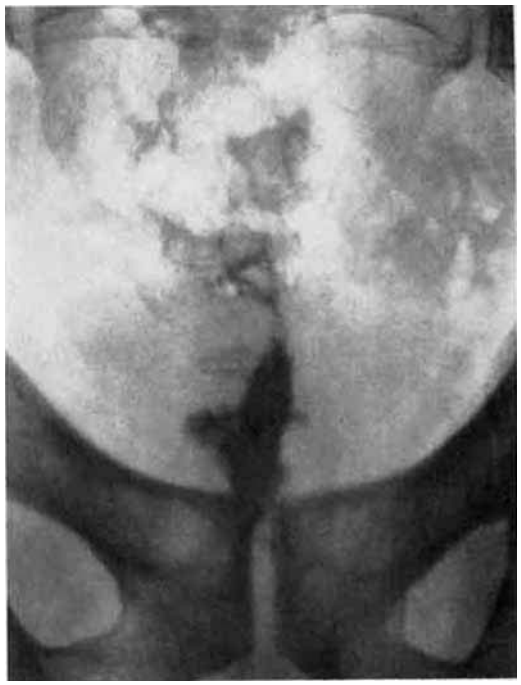

Fig. 6.—Roentgenograph of the pelvic inlet of the same subject as that of Fig. 3, 10 minutes after insertion of a type B suppository. The disintegration of the suppository and the spread of the contrast medium have hardly begun.

When the apparatus is used for the determination of the m.p. of fatty-base suppositories it must be known at what rate the temperature of the suppository comes into equilibrium with that of the apparatus in order to regulate the speed at which the temperature of the apparatus should be raised. For this purpose, suppositories of Imhausen H were prepared and a thermocouple was inserted at the level marked T in Fig. 2 (Imhausen H is a mixture of hydrogenated natural fats, mostly triglycerides of laurinic acid, with a monoglyceric ester as emulsifier). One suppository prepared in this way was put in the apparatus kept at 34.7°. The temperatures measured by the thermocouple in the center of the suppository during 1 hour of experiment are given in Fig. 10*A*.

As the rise in temperature (dT) in the time (dt) is, in all probability, proportional to the difference between the environment temperature (T_e) and the temperature of the suppository (T), the following equation may be written

$$dT/dt = k(T_e - T) \qquad \text{(Eq. 1)}$$

where k is a constant depending on the heat capacity and conductivity of the suppository. By integration, Eq. 1 becomes

$$kt = \log (T_e - T_0)/(T_e - T) \quad \text{(Eq. 2)}$$

where T_0 expresses the temperature of the suppository at the beginning of the experiment.

Equation 2 shows that plotting the logarithm of $(T_e - T_0)/(T_e - T)$ on the ordinate and the time on the abscissa of a diagram of Cartesian axes, a straight line should be obtained with a slope equal

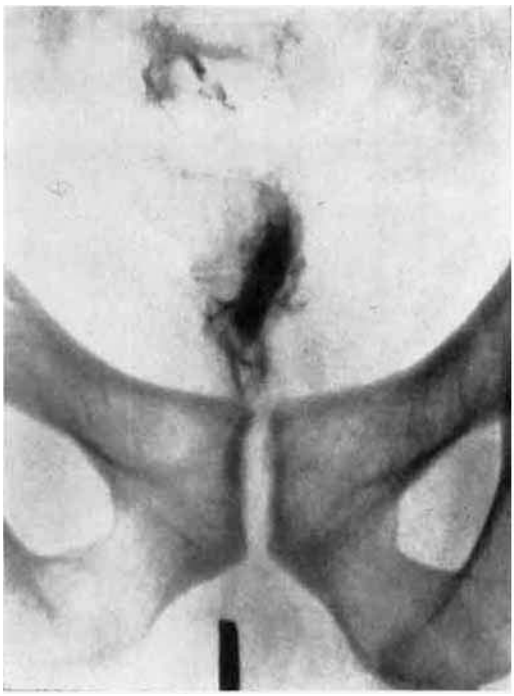

Fig. 7.—Type B suppository, 30 minutes after insertion. The disintegration of the suppository and the spread of the contrast medium are already very evident.

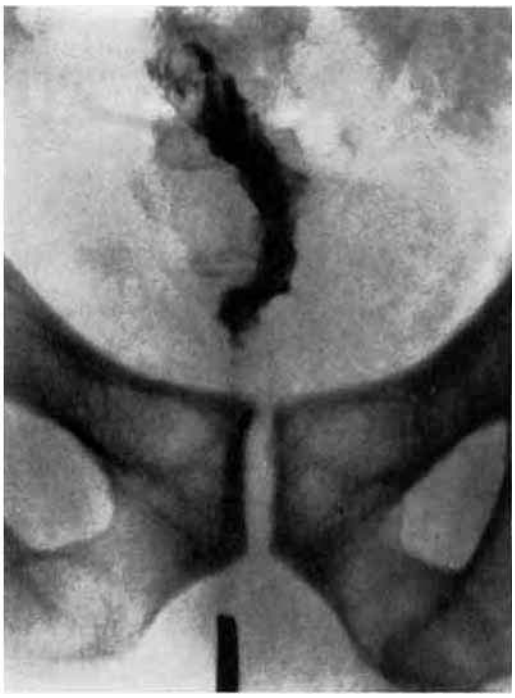

Fig. 8.—Type B suppository, 45 minutes after insertion. Disintegration is complete.

to k. The data of Fig. 10*A* were recalculated in this way (in this experiment T_e was equal to 34.7° and T_0 equal to 18.3°) and the results are reported in Fig. 10*B*.

One notes that the rise in temperature proceeds at first at a higher speed ($k = 0.141$) and then, when in all likelihood a more uniform temperature gradient has formed throughout the thickness of the suppository, the rise proceeds more slowly ($k = 0.053$). The half-equilibrium time is reached in 2 minutes, the 99% equilibrium time in 27.5 minutes.

It may also be calculated that, when the temperature in the apparatus is raised by 1° every 10 minutes, the temperature at the center of the suppository remains behind that of the apparatus by 0.1–0.3°. Under these conditions the error of appreciation of the m.p. is smaller than 0.3°. If one considers that when the suppository begins to melt its diameter is reduced and the transmission of heat is facilitated, the difference between the temperature inside the suppository and that of the environment becomes still smaller and likewise the error.

This method of analysis of the temperature transmission may be useful also in order to know how size, method of preparation, and composition may affect the temperature-equilibrium time and to choose the best conditions from this point of view.

DISCUSSION

In Table I the bases studied are divided into fatty and water-soluble, though the distinction is not always accurate because many commercial bases consist of mixtures of fats and more-or-less water-

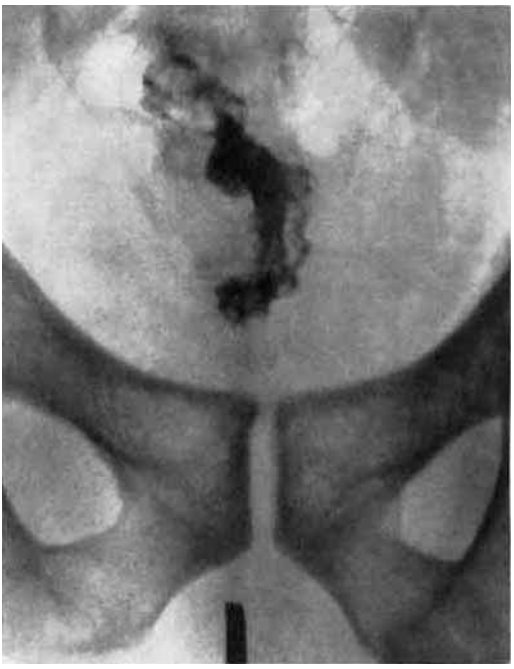

Fig. 9.—Type B suppository, 60 minutes after insertion. Disintegration is complete and the spread of the contrast medium considerable. If this figure is compared with Fig. 5, it will be seen that the spread of the contrast medium is greater. This is probably due to the longer time (15 minutes in Fig. 5 and 60 minutes in Fig. 9). In this figure the contrast medium begins at a distance of about 4 cm. from the anal orifice and extends for about 5 cm. up the rectum. In 60 minutes it has risen on the average about 2 cm. up the rectum.

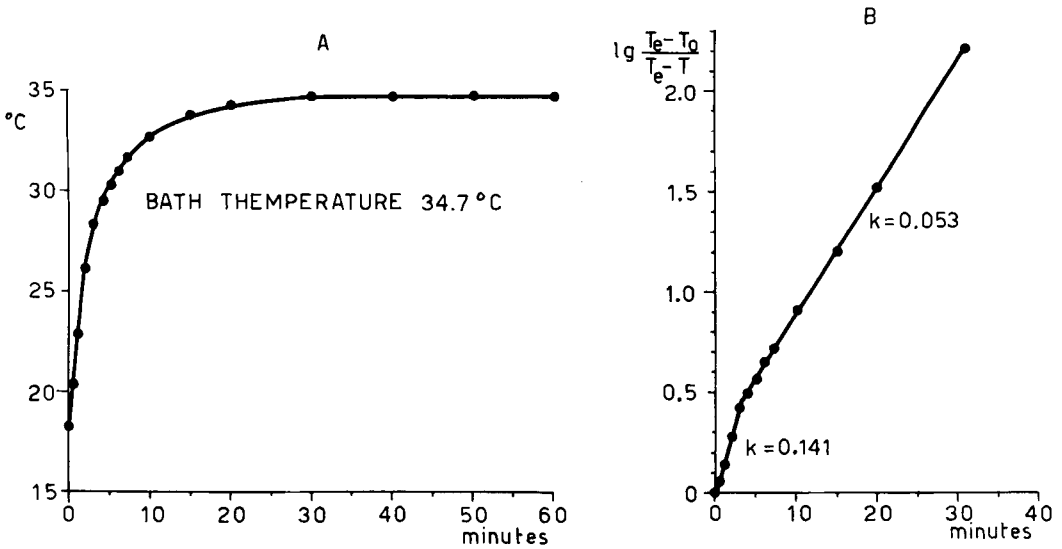

Fig. 10.—Speed of the rise in temperature at the center of the suppository measured with a thermocouple situated in the way described in Fig. 2. The data of Fig. 10*A* are transferred on to Fig. 10*B* to convert Eq. 2 of the text into a linear equation in order to calculate the constant, *k*, of the speed by which the temperature in the center of the suppository increases.

soluble substances which help the fats to emulsify and contribute to the liquefaction process. Indeed, if the base is purely fatty as, e.g., theobroma oil, in order to liquefy it must have a m.p. of 37° or less. This does not happen with Neo Suppostal and Lipomassa C.F.M., which liquefy in the cellophane tube even though they have a m.p. higher than 37°. Therefore, to know whether suppositories liquefy in the rectum, it is not enough to know the m.p. only, even of the so-called fatty bases. Further, as the m.p. of the so-called water-soluble bases is higher than 37°, it must be concluded that, for rectal suppositories, the m.p. tells us very little about the capacity of bases for liquefaction in the rectum.

On the strength of their high m.p., suppositories made with water-soluble bases may, at first sight, seem peculiarly suited to hot or tropical climates. Actually, however, other factors have to be borne in mind, i.e., that liquefaction often takes some considerable time (although it can be shortened by the addition of drugs, propylene glycol, water, etc.) and that these bases dissolve by attracting water from the blood by an antiphysiological process of osmosis which may be irritating and painful. Other disadvantages of these bases have already been noted by Czetsch-Lindenwald (13). Suppositories with water-soluble bases should, therefore, be always subjected to extensive pharmaceutical and biological testing.

We would like also to comment on the definition of suppositories given in the U. S. P. The term "softening" is vague, and the terms "melting" or "dissolving" refer to the base rather than to the suppository, for the drug included does not always melt or dissolve. The reference to "body temperature" is not applicable to dissolving, which depends rather on the presence of water. Further, it seems to us that for rectal suppositories a distinction should be made between those with a local action, e.g., glycerinated gelatin suppositories U. S. P., for which the softening or liquefying is not always essential, and those including a drug for a general action, release of which depends in first instance on the liquefaction of the suppository.

For these suppositories, the liquefaction time is of such great biological importance that a maximum limit should be fixed for it, e.g., 10 minutes, as proposed by Czetsch-Lindenwald (14).

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